Activation of Epidermal Vanilloid Receptor-1 Induces Release of Proinflammatory Mediators in Human Keratinocytes

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ABSTRACT

During dermal injury and the associated trauma a number of compounds are released that can mediate the inflammatory response. Determining the cellular mechanisms that initiate the inflammatory responses to acute keratinocyte damage is important for understanding the regulation of epidermal inflammation. The recently cloned vanilloid receptor-1 (VR1) is a polymodal receptor, responding to thermal, pH, or vanilloids such as capsaicin stimulation. Although VR1 has been localized only on sensory neurons and within the central nervous system, recent evidence suggests a functional VR1 is expressed in human skin and epidermal cells. Using reverse transcription-polymerase chain reaction and immunoblotting we report that human keratinocytes and the human keratinocyte cell line HaCaT express VR1. Consistent with neuronal VR1, activation of epidermal VR1 by capsaicin induced a calcium influx. Treating HaCaT cells with capsaicin resulted in a dose-dependent expression of cyclooxygenase-2 (COX-2), whereas pretreatment with the VR1 receptor antagonist capsazepine abolished the capsaicin-stimulated increase in COX-2 expression. Furthermore, the capsaicin-induced expression of COX-2 was dependent on extracellular calcium. Activation of the epidermal VR1 by capsaicin also resulted in an increased release of interleukin-8 and prostaglandin E2, and the stimulated release was attenuated by capsazepine. The finding that VR1 is expressed by keratinocytes is of great importance because it expands the putative role of VR1 beyond that of pain perception. Our results suggest that VR1 expression in keratinocytes may have a role in the inflammation that occurs secondary to epidermal damage or insult, and thus may function as a sensor for noxious cutaneous stimulation.

Keratinocytes play an active role in the induction of inflammation by synthesizing numerous cytokines and lipid mediators upon injury or insult. Indeed, because of the ability to synthesize and release numerous proinflammatory and trophic mediators, our understanding of the role of keratinocytes in cutaneous inflammation has changed from that of passive bystander to that of an active participant. Human keratinocytes synthesize cytokines such as interleukin-8 in addition to proinflammatory lipid mediators, including platelet-activating factor and prostaglandins (Pentland and Needleman, 1986; Travers et al., 1996; Alappatt et al., 2000). The cellular pathways by which acute keratinocyte damage induces production and release of inflammatory mediators from epidermal cells are largely unresolved, but may involve calcium signaling. Indeed, calcium influx is of primary importance in the expression of inflammatory mediators such as interleukin-8 (IL-8) and in the expression of enzymes, such as cyclooxygenase-2 (COX-2), that synthesize proinflammatory lipid mediators (Bazan et al., 1997; Yu et al., 2001).

The vanilloid receptor-1 (VR1) is a nonselective cationic channel (Oh et al., 1996), and activation of VR1 induces an influx of divalent cations (i.e., Ca2+ and Mg2+). VR1 was recently cloned from both human and rat dorsal root ganglia (Caterina et al., 1997; Hayes et al., 2000). The receptor is comprised of six putative transmembrane domains with both the amino and carboxyl terminus located in the cytoplasm. Consistent with cloned VR1, stimulation of endogenous VR1 on sensory neurons with the vanilloid receptor agonist capsaicin results in dose-dependent influx of Ca2+ (Wood et al., 1988) that is inhibited by the VR1 receptor antagonist capsazepine (Bevan et al., 1992). In addition to VR1 activation by vanilloids, the receptor can be directly activated by exposure to heat or protons (reduced pH), conditions that occur during tissue injury (Tominaga et al., 1998), thus implicating

ABBREVIATIONS: IL-8, interleukin-8; COX-2, cyclooxygenase-2; VR1, vanilloid receptor 1; PGE2, prostaglandin E2; CPAF, 1-hexadecyl-2-N-methyl carbamoyl glycerophosphocholine; AM, acetoxymethyl ester; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; bp, base pair; TPA, 12-O-tetradecanoylphorbol-13-acetate; PAF, platelet-activating factor.
the channel as a primary cellular sensor to thermal or chemical stimulation.

Although VR1 expression has previously been localized to neurons that convey nociceptive transmissions (Szallasi, 1995; Caterina et al., 1997), consistent with the role of VR1 in pain, Denda and coworkers reported on the immunoreactive VR1 expression in human skin and in human keratinocytes (Denda et al., 2001; Inoue et al., 2002). Although VR1 has been localized in the epidermis, the role of VR1 in skin has not been elucidated. Thus, we sought to investigate the function of epidermal VR1 by examining the role of VR1 in inducing expression or release of inflammatory mediators from human keratinocytes. In this study, we report that human keratinocytes and a human keratinocyte cell line, HaCaT, express VR1. Furthermore, activation of VR1 by capsaicin resulted in calcium influx, induced expression of COX-2, and increased release of prostaglandin E2 (PGE2) and IL-8. The VR1 receptor antagonist capsazepine reduced the capsaicin-induced increase in production and release of these inflammatory mediators. Taken together, these results indicate that human keratinocytes express a functional VR1, and activation of epidermal VR1 results in production of proinflammatory mediators. Thus, activation of epidermal VR1 receptor could contribute to the induction of inflammation during noxious cutaneous stimulation.

**Materials and Methods**

**Reagents.** Capsaicin, capsazepine, CPAF, and all other routine chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Capsaicin and capsazepine were initially dissolved in dimethyl sulfoxide to a concentration of 10 mM and then diluted in media or buffer to the appropriate concentration. Capsazepine was used at a concentration of 3 μM, as determined by inhibition of capsaicin responses in sensory neurons (M. R. Vasko, personal communication). In no instances did the vehicle at the dilutions used alter COX-2 expression or inflammatory mediator release. Antiserum for human VR1 and COX-2 proteins was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The calcium-sensitive indicator Fura-2/AM was obtained from Calbiochem (San Diego, CA).

**Cell Culture.** The human keratinocyte cell line HaCaT was grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Intergen, Purchase, NY). Primary cultures of human keratinocytes were obtained from neonatal foreskins as described previously (Kuhn et al., 1999) using Epilife media (Cascade Biologics, Portland, OR).

**Immunoblotting.** Cells were washed twice with ice-cold phosphate-buffered saline and lysed with radioimmunoprecipitation assay buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.1% SDS, 0.5% sodium deoxycholate, 1% Nonidet P-40) containing 0.5 mM Pefabloc SC (Roche Applied Science, Indianapolis, IN), and 10 mM sodium orthovanadate for 20 min on ice. Capsaicin receptor (VR1) or COX-2 expression was determined by immunoblotting with polyclonal anti-VR1 antibody or anti-COX-2 antibody, respectively, and enhanced chemiluminescence (Amersham Biosciences Inc., Piscataway, NJ).

**RNA Isolation and Reverse Transcription-PCR.** Total RNA was isolated from cultured cells using Tripure (Roche Diagnostics, Indianapolis, IN). Following the manufacturer’s procedures of purification, an additional phenol (pH 4.2) extraction was performed, followed by ethanol precipitation. The mRNA was reverse-transcribed into cDNA using Superscript II reverse transcriptase (Superscript II RNase H-Reverse Transcriptase kit; Invitrogen). Briefly, 5 μg of total RNA and 0.5 μg of oligo(dT)12-18 primer were heated to 70°C for 10 min, and briefly chilled on ice. After primer annealing the following were added: 50 mM Tris-HCl, pH 8.8, 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 1 mM dNTP, and 40 units of RNase inhibitor. The reaction incubated for 50 min at 42°C and then for 15 min at 70°C. An aliquot of each reaction was subsequently used as template for a PCR reaction. Primer sequences for human VR1 were as follows: VR1 sense, 5’-ctctcacaacgctgtc-3’; and VR1 antisense, 5’-aaggccagtgtgcaagtcg-3’ (Hayes et al., 2000). The PCR mixture contained a cDNA template derived from total RNA, 1 unit of recombinant TaqDNA polymerase (Invitrogen), 50 pmoles of each of 5′ and 3′ primers (Invitrogen), 0.2 mM dNTP, in a buffer containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgSO₄, in a volume of 50 μl. The PCR reaction for VR1 was performed using a Hybaid PCRExpress thermocycler as follows: 94°C for 120 s and then 30 cycles of 94°C for 45 s, 57°C for 60 s, and 72°C for 120 s followed by 74°C for 10 min. Samples were applied on 1% agarose gel prestained with ethidium bromide.

**Intracellular Calcium Measurements.** HaCaT cells were plated on plastic coverslips (Aclar; Ted Pella, Redding, CA) and allowed to grow for 3 days. The cells were then washed with Ringer’s solution that had the following composition: 140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 10 mM glucose, pH 7.4, adjusted to 7.4 with NaOH. Cells were loaded with the calcium-sensitive indicator Fura-2/AM (5 μM) in Ringer’s solution for 60 min. The Fura-2/AM was diluted from a concentrated stock solution (1 mM) in which the Fura-2/AM was dissolved in dimethyl sulfoxide. After loading, the cells were washed three times with Ringer’s solution and then placed in a recording chamber that could then be superfused with Ringer’s solution. The Fura-2 fluorescence at 340 and 380 nm was measured with an IonOptix recording system (IonOptix, Milton, MA) and changes in calcium were expressed as the ratio of $F_{340} / F_{380}$. In a separate series of experiments, cells loaded with Fura-2 were exposed to 10 μM ionomycin to determine the maximal change in $F_{340} / F_{380}$.

**Measurement of IL-8 and PGE2 Production by Keratinocytes.** Cells were plated at a density of 200,000 cells in 1 ml in 24-well plates for 24 h and then exposed to 0.25% fatty acid-free bovine serum albumin in Hanks’ balanced salt solution (Sigma-Aldrich) with or without drugs. In experiments using antagonists, cells were treated for 30 min before exposure to agonists. The medium was collected 8 h (previously determined) after drug treatment. IL-8 was measured using a Quantikine IL-8 ELISA kit (R&D Systems, Minneapolis, MN) and PGE2 was assayed using a Prostaglandin E₂ EIA kit (Cayman Chemicals, Ann Arbor, MI). After removal of the medium, cells were lysed with 1% sodium dodecyl sulfate and counted to normalize for cell density (Coulter, Hialeah, FL).

**Data Analysis.** Data are presented as mean ± S.E.M. All experiments were performed from three separate batches of keratinocytes. Analysis of variance was used to compare the effects of different treatment on IL-8 and PGE2 release and if a significant difference was observed the Student-Newman-Keuls post hoc test was performed. Student’s t test was used to compare the effects of capsaicin on intracellular calcium. The significance for all tests was set at $P < 0.05$.

**Results**

**Identification of Functional VR1 Receptor in Human Keratinocytes.** To ascertain whether VR1 receptor mRNA is found in human keratinocytes, total RNA from primary cultures of human keratinocytes or the keratinocyte cell line HaCaT was reverse-transcribed and subjected to PCR in the presence of primers for the human VR1 receptor. Figure 1A depicts the PCR products from epidermal cells, corresponding to the VR1 receptor that was detected in the gel at the expected size of 680 bp (Hayes et al., 2000). As the external control, replicate samples of GAPDH PCR product were detected in keratinocytes with an expected size of 600 bp. VR1
A. Human keratinocytes and keratinocyte cell cultures express VR1. A, RNA isolated from normal human keratinocytes (NHK) and a human keratinocyte cell culture (HaCaT) was subjected to reverse transcription-PCR for human VR1 or GAPDH. PCR products were separated on 1% agarose gel and visualized with ethidium bromide. Lanes 1 and 9, 100-bp DNA ladder; lanes 2 and 3, NHK and HaCaT water control; lanes 4 and 5, NHK and HaCaT VR1 or GAPDH PCR product; and lanes 7 and 8, NHK and HaCaT VR1 or GAPDH PCR product treated with Smal. B, VR1 protein expression in NHK and HaCaT cells. Approximately 50 µg of protein was separated on a 10% SDS-polyacrylamide gel electrophoresis, and VR1-immunoreactivity was determined using a polyclonal antibody. Figure is a representative of three experiments with similar results.

PCR product identity was further confirmed by restriction enzyme digestion with Smal to yield two products of 414 and 266 bp, respectively (Fig. 1A). The expression of VR1 receptor protein in keratinocytes was examined using an antibody that recognizes the capsaicin receptor. Immunoreactive VR1 with a molecular mass of approximately 100 kDa was detected when protein isolated from human keratinocytes or HaCaT cells was separated by gel electrophoresis (Fig. 1B). These molecular masses correspond to the sizes of the VR1 receptors (Kuzhikandathil et al., 2001; Yiangou et al., 2001). These results confirm the work of Inoue et al. (2002) that VR1 receptor mediated the induction of COX-2 expression, HaCaT cells were treated with various doses of capsaicin for 4 h and expression of COX-2 was examined. Exposing HaCaT cells to increasing concentration of capsaicin resulted in a dose-dependent increase in COX-2 expression (Fig. 3A); as a positive control, treatment with the platelet-activating factor receptor agonist CPAF resulted in similar induction of COX-2 expression (Pei et al., 1998). To confirm that capsaicin activation of the VR1 receptor mediated the induction of COX-2 expression, HaCaT cells were pretreated with 3 μM VR1 antagonist capsazepine and VR1 agonist capsaicin (1 µM) caused a significant increase in the intracellular levels of calcium (Fig. 2). The resting fluorescence ratio was 0.57 ± 0.02 (n = 13); this value was similar to that observed in sensory neurons isolated from adult rat (L. S. Gharibova and G. D. Nicol, personal communication). Application of 1 µM capsazepine via bath superfusion significantly (paired t test) increased the ratio to 0.81 ± 0.04; the time to peak was variable and ranged from approximately 100 to 200 s of exposure. These increased levels of calcium produced by capsazepine are slightly smaller than those observed in isolated sensory neurons wherein the ratio increased to values between 1.0 and 1.2. In a separate series of experiments, HaCaT cells bathed in Ringer’s solution were exposed to 10 µM ionomycin; application of this calcium ionophore caused a large increase in intracellular levels of calcium that exceeded those produced by capsazepine. These results indicate that the VR1 receptor expressed in HaCaT cells is capable of activation by capsazepine and that activation permits in the influx of extracellular calcium into these cells.

Fig. 1. Human keratinocytes and keratinocyte cell cultures express VR1. A, RNA isolated from normal human keratinocytes (NHK) and a human keratinocyte cell culture (HaCaT) was subjected to reverse transcription-PCR for human VR1 or GAPDH. PCR products were separated on 1% agarose gel and visualized with ethidium bromide. Lanes 1 and 9, 100-bp DNA ladder; lanes 2 and 3, NHK and HaCaT water control; lanes 4 and 5, NHK and HaCaT VR1 or GAPDH PCR product; and lanes 7 and 8, NHK and HaCaT VR1 or GAPDH PCR product treated with Smal. B, VR1 protein expression in NHK and HaCaT cells. Approximately 50 µg of protein was separated on a 10% SDS-polyacrylamide gel electrophoresis, and VR1-immunoreactivity was determined using a polyclonal antibody. Figure is a representative of three experiments with similar results.

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Fig. 2. Activation of epidermal VR1 induces calcium mobilization. The ordinate represents the mean ± S.E.M. of the ratio of f340/f380. HaCaT cells (human keratinocyte cell line) were loaded with 5 µM of the calcium indicator Fura-2/AM for 60 min before the experiment. Cells were washed with Ringer’s solution, followed by bath application of either 1 µM capsaicin or 10 µM ionomycin as a positive control. Fura-2 fluorescence at 340 and 380 nm was determined and changes in calcium were expressed as the ratio of f340/f380. An asterisk indicates a significant difference from control using an unpaired t test (P < 0.05).

Fig. 2A. Activation of epidermal VR1 induces calcium mobilization. The ordinate represents the mean ± S.E.M. of the ratio of f340/f380. HaCaT cells (human keratinocyte cell line) were loaded with 5 µM of the calcium indicator Fura-2/AM for 60 min before the experiment. Cells were washed with Ringer’s solution, followed by bath application of either 1 µM capsaicin or 10 µM ionomycin as a positive control. Fura-2 fluorescence at 340 and 380 nm was determined and changes in calcium were expressed as the ratio of f340/f380. An asterisk indicates a significant difference from control using an unpaired t test (P < 0.05).

Activation of VR1 Induces COX-2 Expression through a Calcium-Dependent Process. To determine whether VR1 activation would induce production of proinflammatory mediators from keratinocytes, HaCaT cells were treated with various doses of capsaicin for 4 h and expression of COX-2 was examined. Exposing HaCaT cells to increasing concentration of capsaicin resulted in a dose-dependent increase in COX-2 expression (Fig. 3A); as a positive control, treatment with the platelet-activating factor receptor agonist CPAF resulted in similar induction of COX-2 expression (Pei et al., 1998). To confirm that capsaicin activation of the VR1 receptor mediated the induction of COX-2 expression, HaCaT cells were pretreated with 3 μM VR1 antagonist capsazepine and VR1 agonist capsaicin (1 µM) caused a significant increase in the intracellular levels of calcium (Fig. 2). The resting fluorescence ratio was 0.57 ± 0.02 (n = 13); this value was similar to that observed in sensory neurons isolated from adult rat (L. S. Gharibova and G. D. Nicol, personal communication). Application of 1 µM capsazepine via bath superfusion significantly (paired t test) increased the ratio to 0.81 ± 0.04; the time to peak was variable and ranged from approximately 100 to 200 s of exposure. These increased levels of calcium produced by capsazepine are slightly smaller than those observed in isolated sensory neurons wherein the ratio increased to values between 1.0 and 1.2. In a separate series of experiments, HaCaT cells bathed in Ringer’s solution were exposed to 10 µM ionomycin; application of this calcium ionophore caused a large increase in intracellular levels of calcium that exceeded those produced by capsazepine. These results indicate that the VR1 receptor expressed in HaCaT cells is capable of activation by capsazepine and that activation permits in the influx of extracellular calcium into these cells.

Fig. 3A. Activation of human keratinocytes with 10 nM and 1000 nM CPAF for 6 h. After treatment COX-2 expression was examined. C, HaCaT cells were treated with 1 µM capsazepine or 100 nM CPAF, or 1 µM TPA for 6 h in normal buffer or buffer deficient in calcium. After treatment COX-2 expression was examined.

Fig. 3B. Activation of epidermal VR1 induces COX-2 expression. A, HaCaT cells were treated with various doses of the VR1 agonist capsaicin or with PAF receptor agonist (CPAF, 100 nM) as a positive control for 6 h. Approximately 50 µg of protein was separated on a 10% SDS-polyacrylamide gel electrophoresis, and COX-2-immunoreactivity was determined using a polyclonal antibody. B, HaCaT cells were pretreated for 30 min with a VR1 receptor antagonist capsazepine (CZP, 3 µM) before stimulation with 1 µM capsaicin or 100 nM CPAF for 6 h. After treatment COX-2 expression was examined. C, HaCaT cells were treated with 1 µM capsazepine or 100 nM CPAF, or 1 µM TPA for 6 h in normal buffer or buffer deficient in calcium. After treatment COX-2 expression was examined.

Fig. 3C. Activation of epidermal VR1 induces COX-2 expression. A, HaCaT cells were treated with various doses of the VR1 agonist capsaicin or with PAF receptor agonist (CPAF, 100 nM) as a positive control for 6 h. Approximately 50 µg of protein was separated on a 10% SDS-polyacrylamide gel electrophoresis, and COX-2-immunoreactivity was determined using a polyclonal antibody. B, HaCaT cells were pretreated for 30 min with a VR1 receptor antagonist capsazepine (CZP, 3 µM) before stimulation with 1 µM capsaicin or 100 nM CPAF for 6 h. After treatment COX-2 expression was examined. C, HaCaT cells were treated with 1 µM capsazepine or 100 nM CPAF, or 1 µM TPA for 6 h in normal buffer or buffer deficient in calcium. After treatment COX-2 expression was examined.
(Dickenson and Dray, 1991; Szallasi et al., 1999), for 30 min before and throughout the 4-h treatment with capsaicin. As shown in Fig. 3B, exposure to capsazepine attenuated the capsaicin-induced expression of COX-2 in keratinocytes. Exposure to capsazepine alone did not affect COX-2 expression. In addition, pretreatment with capsazepine did not affect the CPAF-induced COX-2 expression (Fig. 3B). To evaluate the role of extracellular calcium in capsaicin-induced COX-2 expression, HaCaT cells were treated with capsaicin, CPAF, or the phorbol ester TPA in buffer deficient in calcium. As illustrated in Fig. 3C, capsazepin, CPAF, and TPA induced COX-2 expression in buffer containing calcium; however, the capsazepin- and CPAF- but not TPA-induced COX-2 expression was abolished in cells treated with buffer deficient in extracellular calcium. Taken together, these results indicate that activation of VR1 mediates the capsaicin-induced COX-2 expression through a calcium-dependent manner.

VR1 Activation Induces PGE2 Release. Keratinocytes have been shown to synthesize PGE2 in response to various proinflammatory stimuli, including tumor necrosis factor-α, PAF, and ultraviolet B radiation (Pei et al., 1998; Countryman et al., 2000). To address whether activation of VR1 would induce PGE2 release from keratinocytes, HaCaT cells were treated with 1 μM capsaicin or 100 nM CPAF and PGE2 release was determined. As shown in Fig. 4, treatment of HaCaT cells with capsaicin induced PGE2 release and this release was inhibited by pretreatment with 3 μM VR1 antagonist capsazepine. Exposure to CPAF also resulted in an increase in PGE2 release; however, the CPAF-mediated release of PGE2 was unaffected by capsazepine. Taken together, these results demonstrate that activation of the epidermal VR1 results in COX-2 expression and the subsequent release of PGE2. Furthermore, the actions of capsaicin were shown to be secondary to activation of VR1.

VR1 Activation Induces IL-8 Release. Keratinocytes produce IL-8 in response to numerous stimuli, including proinflammatory cytokines and to PAF (Pei et al., 1998; Countryman et al., 2000). To determine whether activation of VR1 would similarly induce IL-8 release from keratinocytes, HaCaT cells were treated with 1 μM capsaicin or 100 nM CPAF and IL-8 release was determined. As shown in Fig. 5, incubation of HaCaT cells with capsaicin induced IL-8 release in a manner similar to release stimulated with CPAF. Pretreatment with 3 μM VR1 antagonist capsapezpine resulted in an inhibition of the capsaicin-induced IL-8 release without affecting the CPAF-induced release (Fig. 5).

Discussion

Although the expression of VR1 receptors has been well established in sensory neurons (Szallasi, 1995; Mezey et al., 2000), the existence of VR1 in keratinocytes is controversial. Previous studies indicated that VR1 expression in skin was localized on the terminals of afferent neurons at the dermal/epidermal junction (Guo et al., 1998). Several lines of evidence indicate other cell types, including keratinocytes, express a functional VR1. First, VR1 has recently been identified in cardiomyocytes (Dvorakova and Kummer, 2001), bronchial epithelial cells (Veronesi et al., 1999b), and urinary bladder epithelial cells (Birder et al., 2001) independent of sensory neurons, thus establishing that non-neuronal cells can express VR1. Keratinocytes express receptors that were previously thought to be confined to neuronal cells, including nicotinic (Grando et al., 1995), muscarinic (Ndoye et al., 1998), and μ-opiate (Bigliardi-Qi et al., 1999), although the functional role for many of these receptors has yet to be elucidated. In addition, Denda and coworkers reported that immunoreactive VR1 was present on epidermal keratinocytes in human skin (Denda et al., 2002; Inoue et al., 2002). Exposing cultured keratinocytes to capsaicin induces cell death (Ko et al., 1998); whereas cell death does not occur in
cells lacking VR1 (Caterina et al., 1997). And finally, using reverse transcription-PCR, immunoblotting, and activity assays, our study has demonstrated that a functional VR1 is present in human keratinocytes.

Although the lipid mediator capsaicin can pass through cellular membranes and thus act on intracellular proteins (Jung et al., 1999), studies suggest that activation of the epidermal VR1 mediates the proinflammatory response to capsaicin. They include treatment with capsaicin has no effect on cellular responses in VR1-deficient cells (Caterina et al., 1997) and site-directed mutagenesis of VR1 inhibits activation by capsaicin (Kuzuhikandathil et al., 2001), indicating that receptor expression is of primary importance for vanilloid activity. Also, inhibition of epidermal VR1 with the VR1 receptor antagonist capsazepine (Dickenson and Dray, 1991; Bevan et al., 1992; Szallasi et al., 1999) attenuated the effects of capsaicin stimulation on COX-2 expression and the release of IL-8 and PGE₂. In addition, capsazepine did not affect the keratinocyte response to PAF receptor stimulation, further suggesting the inhibitory effects of capsazepine were specific to VR1 receptor. Thus, activation of the epidermal VR1 induces eicosanoid formation and release cytokines in a manner similar to other proinflammatory mediators such as PAF.

Our results indicate that the capsaicin-induced expression of proinflammatory mediators by keratinocytes occurs through a calcium-dependent pathway because exposure of cultured keratinocytes to capsaicin in the absence of extracellular calcium abolished the expression of COX-2. VR1 is a nonselective cationic channel that conducts calcium ions (Oh et al., 1996; Nagy and Rang, 1999) and capsaicin-induced activation of the epidermal VR1 results in a similar calcium conductance (Fig. 2). COX-2 expression by epithelial cells requires an increase in intracellular calcium (Guo et al., 2001) and this expression can be mimicked with calcium ionophores or inhibited by removal of extracellular calcium (Bazan et al., 1997). In addition, treatment of human airway epithelial cells with capsaicin induces IL-8 production in a calcium-dependent manner (Veronesi et al., 1999a). Thus, the activation of epidermal VR1 and the subsequent elevation of intracellular calcium are of primary importance in the vanilloid-mediated production of proinflammatory mediators.

Topical or intradermal injection of capsaicin has been shown to induce edema (Buckley et al., 1990), but these inflammatory responses have been generally attributed to the capsaicin-stimulated release of neuropeptides, predominantly substance P, from sensory neurons (Inoue et al., 1993, 1995). This is supported by observations that plasma extravasation, an indication of neurogenic inflammation, does not occur when capsaicin is applied to the denervated skin of the rat hindpaw (Janse et al., 1967). The present findings, however, demonstrate that keratinocytes are a direct target of capsaicin through activation of an epidermal VR1; yet, the role that the VR1-induced release of proinflammatory mediators has in the development of an inflammatory response is unclear. It is interesting to speculate that the production and release of inflammatory mediators produced by keratinocytes secondary to activation of the epidermal VR1 could act on terminals of sensory neurons in the skin (Treede et al., 1992), and thus augment neurogenic inflammation and nociceptive signaling. Indeed, PGE₂ enhances the capsaicin-stimulated release of substance P from sensory neurons (Southall and Vasko, 2001) and inhibitors of prostaglandin synthesis block capsaicin-induced edema (Gamillscheg et al., 1984; Gabor and Razga, 1992). A similar role was proposed for VR1 in urinary epithelial cells, which involves the release of mediators from urinary epithelial to regulate activity of nerves in the bladder wall (Bird et al., 2001). Further studies are warranted to determine the role of epidermal VR1 activation and release of proinflammatory mediators on the development of inflammation.

Taken together, our results support that notion that the epidermal VR1 may function as a sensor for noxious stimuli in the skin. VR1 is activated by diverse agents, including heat, protons, particulate matter, and vanilloid compounds such as capsaicin (Caterina et al., 1997; Tominaga et al., 1998; Veronesi et al., 1999b). Other proinflammatory mediators, such as leukotrienes, are reported to be weak activators of VR1, acting through direct actions on VR1 (Hwang et al., 2000). Our laboratory has previously demonstrated that acute thermal stimulation of keratinocytes induces production of inflammatory mediators from keratinoctyes (Alappat et al., 2000), although it remains to be seen whether this response is mediated by VR1. Keratinocytes responses to numerous environmental stimuli may therefore be mediated by activation of VR1, further displacing the notion that keratinocytes function strictly in a barrier role for the body.

References
Hayes P, Meadows HJ, Gunthorpe MJ, Harries MH, Duckworth DM, Cairns W,